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HEALTH EFFECTS DIVISION  
SCIENTIFIC DATA REVIEWS  
EPA SERIES 361

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UNITED STATES ENVIRONMENTAL PROTECTION AGENCY  
WASHINGTON, D.C. 20460

OFFICE OF  
PREVENTION, PESTICIDES AND  
TOXIC SUBSTANCES

DATE: July 9, 2003

MEMORANDUM

TXR No. 0052006

SUBJECT: Mechanism of Toxicity SARC Second Report: *Acifluorfen* (PC Code: 114402)

FROM: Paul Chin *Paul Chin*  
Reregistration Branch I  
Health Effects Division (7509C)

TO: Pauline Wagner, Co-Chair *Pauline Wagner 7/10/03*  
Mechanism of Toxicity Assessment Review Committee (MTARC)  
Health Effects Division (7509C)  
and  
Karl Baetcke, Co-Chair *Karl Baetcke*  
Mechanism of Toxicity Assessment Review Committee (MTARC)  
Health Effects Division (7509C)

cc: Anna Lowit (RRB2), Kit Farwell (RRB1), Christina Scheltema (SRRD)

The Mechanism of Toxicity Assessment Review Committee (MTARC) reviewed the toxicological data supporting peroxisome proliferation as a proposed mode of action for the carcinogenic effects of acifluorfen in mice on April 17, 2003. The first report contains the conclusions from this meeting.

At the recent Cancer Assessment Review Committee (CARC) meeting (May 21, 2003), the CARC concluded that the increased levels of enzymes (ALP and SGPT) seen at 40.5 mg/kg/day in the carcinogenicity study in mice (CD-1) were considered equivocal because of large standard deviations. Therefore, the first MTARC report was revised to reflect this conclusion.

**THIS DOCUMENT SUPERSEDES THE PREVIOUS MTARC REPORT DATED MAY 14, 2003 (TXR No. 0050227).**



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### Committee Members in Attendance

Members who were present and gave electronic concurrence to this report were: Vicki Dellarco, Alberto Protzel, Anna Lowit, Ayaad Assaad, Karen Hamernik, Karl Baetcke, Pauline Wagner, Elizabeth Mendez, and John Doherty.

Also in attendance were: Whang Phang (RRB1), Michael Metzger (RRB1), Kit Farwell (RRB1), Tawanda Spears (SRRD), Brian Dementi (TOX)

Data Evaluation / Report Preparation

  
Byong-Han Chin, Ph.D  
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Toxicologist

## I. Conclusion

The Mechanism of Toxicity Assessment Review Committee (MTARC) reviewed and debated the toxicological data supporting peroxisome proliferation as a proposed mode of action for the carcinogenic effects of acifluorfen in mice. The MTARC concluded that the currently available data are considered to be sufficient to support peroxisome proliferation as the mode of action of acifluorfen-induced liver tumors in mice according to the criteria recommended by International Life Sciences Institute (ILSI); these include:

1. Changes in liver morphology were observed in both rats and mice treated with acifluorfen. These effects include: dose-dependent increase in relative liver weights, and increased incidence of cellular hypertrophy, and increased number of peroxisomes.
2. Evidence of cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling in light microscopy.
3. Dose-dependent increase in the levels of CN-insensitive acyl (palmitoyl) CoA oxidase activities involved in peroxisomal fatty acid metabolism.

Previously, the MTARC concluded that the peroxisome proliferation is the mode of action of lactofen in inducing liver tumors in rodents. Since acifluorfen is the major metabolite of lactofen in the rodents, acifluorfen is considered likely to contribute to the peroxisome proliferation induced by lactofen. Therefore, the data on lactofen provide some support for acifluorfen.

## II. Background Information

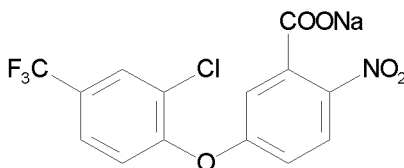
ACIFLUORFEN (Sodium 5-[2-chloro-4-(trifluoromethyl) phenoxy]-2-nitrobenzoate) is the active ingredient of two herbicides formulations, Tackle and Blazer, which were originally manufactured by two separate companies. Acifluorfen is a contact herbicide used for selective pre- and post-emergence control of annual broad-leaf weeds and some grasses in rice, peanut, and soybean production. Acifluorfen is also a major metabolite of another registered herbicide lactofen.

Although toxicological data are available on both acifluorfen products, the data provided on Blazer are incomplete, whereas, the data on Tackle are more current and complete. Therefore, discussions of the toxicology of acifluorfen are predominantly based on the data derived from the more recent studies with Tackle, and where appropriate the data on Blazer are also utilized. It should be noted that Tackle contains about 20% to 24% of acifluorfen as the active ingredient, whereas Blazer contains approximately 40% of acifluorfen.

In rats, acifluorfen was rapidly absorbed following oral administration and eliminated mainly in the urine (46-58%) and feces (21-41%). The major component present in urine was unchanged acifluorfen and the major component in feces was unchanged acifluorfen and an amine metabolite. No tissue accumulation was observed.

### III. Chemical and Physical Properties of Acifluorfen

Chemical Structure:



Common Synonyms: Blazer, Tackle

Physical Properties:

Melting point: >212° F

Water Solubility: >25%

CAS No.: 62476-59-9

### IV. Regulatory History

The registrant (BASF) submitted a petition (MRID No. 45323500) requesting that risk assessment for acifluorfen be based on the MOE approach rather than using a  $Q_1^*$  in human equivalents (from K. Blundell, BASF to Ms. Christina Scheltema, Chemical Review Manager, SRRD, MRID No. 45323500 dated Feb. 2, 2001). The petition reviewed and summarized earlier data submissions which supported peroxisome proliferation as the mode of action of acifluorfen for induction of liver tumors. The current unit risk [ $Q_1^*$ ] is  $1.27 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$  (memorandum from L. Brunsman to P. Chin, dated November 8, 2001).

The data supporting the proposed mode of action as presented by the registrants were considered at a preliminary meeting of the Mechanism of Toxicity Assessment Review Committee (MTARC), HED on Aug. 23, 2001. The Committee evaluated the available data according to criteria recommended by the International Life Sciences Institute (ILSI) workshop on peroxisome proliferation for determining whether or not liver tumors were induced by acifluorfen via a peroxisomal proliferation. The ILSI criteria are as follows:

1. Changes in liver morphology indicating hepatomegaly as measured by increased relative liver weights and an increased number of peroxisomes as measured by morphometric analysis.

2. Evidence of cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling in light microscopy.
3. Increased levels of enzymes involved in peroxisomal fatty acid metabolism, especially CN-insensitive acyl (palmitoyl) CoA oxidase activities.

The MTARC concluded that the available data were insufficient to support the proposed mode of action of peroxisome proliferation for acifluorfen. BASF was informed of this decision (e-mail to R. Hawks from B. H. Chin, dated 9/12/01). Subsequently the registrant proceeded to develop the necessary data and submitted the following new mechanism studies with acifluorfen:

- 1) The induction of the number and size of hepatic peroxisomes in mice following 4 week dietary feeding with acifluorfen (MRID 45693401),
- 2) S-phase response study in the liver of mice following 3 days, 1 week, and 2 weeks feeding with acifluorfen (MRID45803601)
- 3) Enzyme induction study in the liver of mice following 4 weeks feeding with acifluorfen (MRID 45793901).

## **V. Evaluation of the Toxicology Data Base for Acifluorfen**

### **Subchronic Toxicity Studies with acifluorfen**

Available studies are adequate to satisfy subchronic testing requirements for acifluorfen. The subchronic feeding studies in rats and mice showed decrease in body weight and liver toxicity such as increased liver weight and increased incidence of cellular hypertrophy. There is no subchronic toxicity in dogs on acifluorfen. However, there was an acceptable chronic feeding toxicity study in dogs.

### **Chronic Toxicity and Carcinogenicity Studies with acifluorfen and Classification of Carcinogenic Potential**

The chronic feeding toxicity studies in rats, mice and dogs demonstrated that acifluorfen induced liver toxicity (acidophilic cells in the liver and increased liver weight) and kidney toxicity (nephritis/pyelonephritis and increased kidney weight). Acifluorfen induces an increase in combined malignant and benign liver tumor incidence in two mouse carcinogenicity studies employing different strains of mice (B6C3F1 and CR-CD-1) [HED Doc. No. 003410; 001099; 003963; 003409; 003556]. The data were evaluated by the HED Cancer Peer Review Committee (HED Doc. No. 007698 dated March 17, 1988). Acifluorfen was classified as a Group B, probable human carcinogen, and the unit risk [ $q^*_1$ ] was calculated to be  $1.27 \times 10^{-2} \text{ (mg/kg/day)}^{-1}$  (memorandum from L. Brunsman to P. Chin, dated November 8, 2001). A two-year bioassay in the F-344 rat

administering Tackle (approximately 20-24% acifluorfen) in the diet for 24 months did not show any treatment-related increase in tumor incidence.

### **Mutagenicity**

The genetic toxicology studies indicate that acifluorfen was weakly mutagenic in *Salmonella typhimurium* TA100 and in *Saccharomyces cerevisiae* in the presence of S9 metabolic activation. However, in a repeat study, acifluorfen was negative in *Salmonella typhimurium* strains TA98, TA100, TA1535, and TA1537 in the presence and absence of metabolic activation. Also, the test material was negative for gene mutations in Chinese hamster ovary (CHO) cells and for clastogenic effects *in vivo*. In addition, acifluorfen was not mutagenic in DNA synthesis assay in primary rat hepatocytes and in *in vivo* dominant lethal assay in rats. The mutagenicity studies satisfy the pre-1991 mutagenicity guideline requirements. Summaries of the submitted acceptable mutagenicity studies are presented below (Table 1).

### **Mechanism Studies with acifluorfen**

The relevant data concerning possible acifluorfen induced peroxisome proliferation are presented as follows:

#### **A. Liver Toxicity (Table 2)**

Pertinent results from various guideline studies that are relevant to the proposed mechanism of action of acifluorfen are summarized in Table 2. There are several studies that show acifluorfen induced liver toxicity. The subchronic feeding toxicity studies in rats and mice, chronic feeding toxicity studies in rats and dogs, and carcinogenicity study in mice (CD-1 and B6C3F1) showed that acifluorfen caused liver toxicity as characterized by increased absolute and relative liver weights. In addition, acifluorfen caused increased microscopic change in the liver in the chronic rat study at 125 mg/kg/day (increased incidence of acidophilic cells) and in the chronic dog study at 112.5 mg/kg/day (congestion, brown pigment, fatty vacuoles and inflammation). In the 90-day study in B6C3F1 mice, fatty infiltration of the liver was observed at 187.5 mg/kg/day or higher and increased incidence of histopathological changes in the liver (hypertrophy, increased mitotic activity, and oval cell proliferation) and increased levels of enzymes (ALP and SGPT) were seen at 375 mg/kg/day or higher. These levels were above the dose levels which induce peroxisome proliferation. It is noted that increased levels of enzymes (ALP and SGPT) seen at 40.5 mg/kg/day in the carcinogenicity study in mice (CD-1) were considered equivocal because of large standard deviations.

#### **B. Peroxisome Proliferation (Table 3)**

A study designed to determine whether or not acifluorfen induces an increase in peroxisome proliferation is available and the executive summary of the study follows:

The effects of 4-week dietary administration of Blazer Technical (Lot # 01501L300, purity 46% acifluorfen) on the induction of the number and size of hepatic peroxisomes in male and female B6C3F1 mice (5/sex/dose) was investigated (MRID 45693401). The administered concentrations were 0, 350, 1735, or 5210 ppm (equivalent to 0, 92, 484, and 1346 mg/kg/day in males and 0, 139, 644, and 1939 mg/kg/day in females, respectively). Mean **daily intakes of the a.i.** were 42/64, 223/296 and 619/892 mg/kg/day, [males/females], respectively. Blazer Technical induced a slight to moderate increase in the number of peroxisomes within the centrilobular region of the high-dose group of both sexes observable by light microscopy. No changes were found in male and female mice of lower dose groups. By electron microscopy, treatment with Blazer Technical induced a dose-related increase in the number, size, and area of hepatic peroxisomes in mice treated with  $\geq 350$  ppm test material, thereby suggesting that **Blazer Technical is a weak peroxisome proliferator**. At 350 ppm, only slight increase of size 1 peroxisome (up to 1.5 fold;  $p < 0.05$ ) in males and females were observed.

At 1735 ppm, significant increase in number of peroxisomes (2-6 fold;  $p < 0.01$ ) in both sexes were observed. In both sexes of mice, dose-related significant increase ( $p < 0.01$ ) in total peroxisomal area was apparent (2.7 fold increases in males and 2.2 fold increases in females).

At 5210 ppm, significant increase in number of peroxisomes in males (3-23 fold;  $p < 0.01$ ) and females (1.5-35 fold;  $p < 0.01$ ) were observed. In both sexes of mice, dose-related significant increase ( $p < 0.01$ ) in total peroxisomal area was apparent (7.2 fold increases in males and 8.3 fold increases in females).

By electron microscopy, the positive control diisononyl phthalate (DINP) treatment for 4 weeks in mice significantly increased the size, number, and cytoplasmic area of peroxisomes in male mice treated with  $\geq 500$  ppm and female mice treated with  $\geq 1500$  ppm. Particularly increased were the number of microperoxisomes in mice treated with  $\geq 4000$  ppm DINP. There were also a dose-related increase in cytoplasmic volume of male and female mice treated with  $\geq 1500$  ppm positive control (**MRID 45686501**).

### **C. Evidence of Cell Proliferation (Table 3)**

A study designed to determine whether or not acifluorfen induces cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling is available. The executive summary of the study follows:

In a DNA synthesis (S-phase response)/cell proliferation study (MRID 45803601), Blazer Technical (46.1% sodium acifluorfen in acetone) was administered to groups of 8 male and 8 female B6C3F1 mice at dietary concentrations of 0, 350, 1735 and 5210 ppm (*i.e.*: 160, 800 and 2400 ppm of the active ingredient), mean daily intakes of the a.i. for males/females of 40/54, 227/287 and 714/845 mg/kg/day, respectively, for 3 days, 1 week or 2 weeks. Food consumption and body weights were determined weekly. The animals were examined

at least once a day; and additionally, comprehensive clinical examination was performed weekly. One week prior to necropsy, osmotic mini-pumps containing bromodeoxyuridine (BrdU) were implanted subcutaneously. Cell proliferation (S-phase response) and apoptosis were determined in the liver.

No animals died during the study and no clinical signs were observed. The test material had no effect on food or water consumption, and there were no consistent effects on male or female body weight.

Dose-related increases in liver weights (generally both absolute and relative) and findings of moderate or moderate to severe increases in panlobular hypertrophy of hepatocytes were seen in males and females exposed to 714/845 a.i. mg/kg/day, respectively, on all treatment schedules (3-day, 1- and 2-week). Liver hypertrophy was most pronounced in the males and peaked after 1 week of treatment. By 2 weeks, an increase in single cell necrosis and apoptotic cells was observed primarily in high-dose males. This finding, suggesting cytotoxicity to the target organ, indicates that dosing was adequate to assess cell proliferation in the liver. **The oral administration of Blazer Technical (46.1% acifluorfen-sodium) to mice produced a dose-dependent and significant induction of BrdU labeling in the liver, which is indicative of cell proliferation (S-phase response), in all dose groups. The most pronounced effect for each of these three parameters (↑liver weight, ↑liver hypertrophy and ↑BrdU labeling) for both sexes was seen after 1 week of treatment.**

This study is classified as acceptable (non-guideline). Although this study does not satisfy the requirement for any current FIFRA Test Guideline 84-2, the results may be used in a possible mode of action analysis of the test substance.

#### **D. Evidence of Increased Enzymatic Activity (Table 3)**

A study designed to determine whether or not acifluorfen induces enzymes involved in peroxisomal fatty acid metabolism, especially CN-insensitive acyl (palmitoyl) CoA oxidase activities is available. The executive summary of the study follows:

In an enzyme induction study (MRID 45793901), Blazer technical (46.1% acifluorfen-Na a.i. in acetone) was administered to groups of 10 male and 10 female B6C3F1 mice at dietary concentrations of 0, 350, 1735 and 5210 ppm (160, 800 and 2400 ppm of active ingredient) for 4 weeks. Mean daily intakes of the a.i. were 36.9/54.6, 180/255 and 709/933 mg/kg/day, [males/females], respectively. Food consumption and body weights were determined weekly. Animals were examined once a day, and a comprehensive clinical examination carried out weekly. In the first 5 animals of each group, glutathione concentration (GSH) in the liver was determined; in the second 5 animals, the amount of cyanide-insensitive palmitoyl-CoA-oxidation (PALCoA) in total protein was measured.

At the high-dose, significant ( $p \leq 0.01$ ) decreases in male body weights were recorded on days 14, 21 and 28; the greatest decrease in male body weights (9.2%) occurred on Day 28.

Body weight gain for this group was also significantly ( $p \leq 0.01$ ) reduced compared to control at the same time intervals. No clear adverse effects were found on female body weight. There was a significant ( $p \leq 0.01$ ) and dose-dependent increase in PALCoA, which is involved in peroxisomal fatty acid metabolism, ranging from a 58 to 576% increase for males and a 3 to 707% increase for females at 350 to 5210 ppm, respectively. Increased PALCoA was accompanied by decreased GSH concentrations in high-dose males (9% ↓) and females (15% ↓); the response was dose related for both sexes but only reached statistical significance in the high-dose females. **Based on these considerations, it was concluded that Blazer technical, containing 46.1% NA acifluorfen as the a.i., showed clear evidence of the induction of the peroxisomal enzyme system.** Thus, the demonstration of peroxisomal enzyme system induction in the liver satisfies one of the necessary criteria to classify a nongenotoxic substance as a peroxisome proliferator.

The following special study also supports the findings of the new mechanism study described above. In this special study, hepatocytes isolated from male Sprague-Dawley rats were exposed to acifluorfen or lactofen (MRID No.: 45283902) to examine the induction of palmitoyl CoA oxidase activity following *in vitro* exposure. This study showed that both acifluorfen and lactofen displayed increased activity of palmitoyl CoA oxidase activity following *in vitro* exposure (**Table 3**).

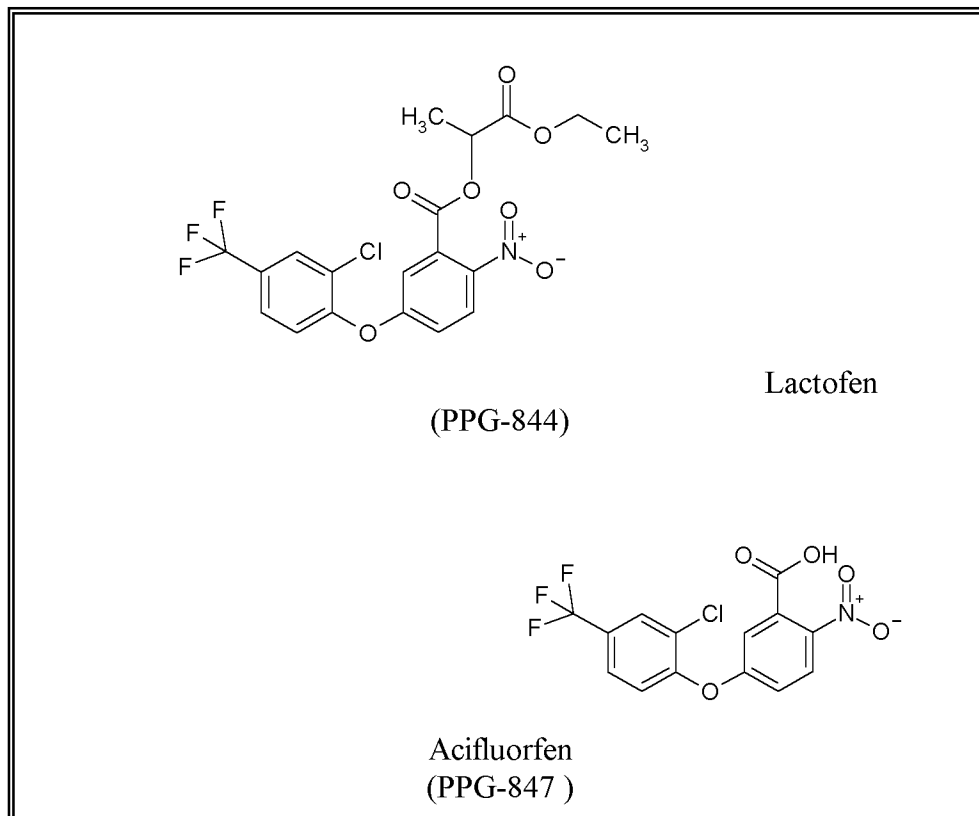
## VI Data from Lactofen Studies

The relevance of peroxisome proliferation (PP) to hepatocarcinogenesis has been previously discussed for lactofen (see HED Memorandum: Lactofen: Report of the Mechanism of Toxicity Assessment Review Committee from Robert F. Fricke to Christine Olinger, dated March 12, 2001).

### A. Rationale for Using Lactofen Data

1. Lactofen is a diphenyl ether herbicide and the structure of lactofen is very similar to that of acifluorfen (see below).
2. The metabolism study of lactofen in rats showed that acifluorfen is a major metabolite of lactofen (Accession No. 071222). Seventy two hours after administration of lactofen to rats,  $\geq 97\%$  of the radiolabel was recovered in the excreta (urine and feces). Urinary excretion comprised 39 - 56% of the dose while the fecal output totaled  $\sim 43$  - 67% of the dose. While the parent compound, lactofen, was the major component in the feces, the major metabolite in urine was acifluorfen which accounted for  $> 90\%$  of the radioactivity recovered in this fraction.

**Figure 1: Structures of Lactofen and Acifluorfen**



3. Following a single oral dose of radiolabeled lactofen at 50 mg/kg to the SD rats and mice (strain not identified), acifluorfen was the primary metabolite of lactofen in the plasma. The plasma level of acifluorfen at peak ranged 61-71% of the administered radioactivity [Hawks, (2001). p22 of the BASF Report dated January 31, 2001 entitled Sodium Acifluorfen: Request for Reassessment of Current Cancer Classification Considering Peroxisome Proliferation–Associated Mode of Action].

**B. The Lactofen Data in Supporting That the Peroxisome Proliferation is the Mode of Action of Lactofen**

The submitted lactofen data in supporting that peroxisome proliferation is the mode of action of lactofen are as follows:

1. The mutagenicity studies showed that lactofen is neither mutagenic nor genotoxic. A non-guideline mutagenicity study showed equivocal (probably negative) binding of lactofen to DNA.

2. Changes in liver morphology were observed in both rats and mice treated with lactofen in the diet for 8 and 7 weeks, respectively (MRID No. 45283904). These effects include: dose-dependent increase in relative liver weights and increased number of peroxisomes as measured by electron microscopic analysis. Further, in both rats and mice there was a dose-dependent increase in nuclear enlargement, cytoplasmic eosinophilia, hypertrophy and peroxisomal staining. In addition, treatment with lactofen resulted in dose-dependent increases (particularly in female mice) in the activities of hepatic CN-insensitive palmitoyl CoA oxidase and carnitine acetyl transferase.
3. Dose-dependent increase in CN-insensitive palmitoyl CoA oxidase was also observed in primary rat hepatocytes treated with lactofen (MRID No. 45283902).
4. The doses at which carcinogenicity were observed [mouse, LOAEL = 50 ppm (7.1 mg/kg/day); rat, LOAEL = 2000 ppm (76 mg/kg/day)] were consistently higher than the doses which caused peroxisome proliferation (for details see HED Memorandum: Lactofen: Report of the Mechanism of Toxicity Assessment Review Committee from Robert F. Fricke to Christine Olinger, dated March 12, 2001).

The major metabolite of lactofen in the rodent is acifluorfen (see **Table 4. Comparative Metabolism of acifluorfen and lactofen in Rats**). Since the MTARC concluded that the peroxisome proliferation is the mode of action of lactofen, peroxisome proliferation data generated using lactofen can be considered in evaluating the hypothesis that acifluorfen is a peroxisome proliferation.

## VII. Other Modes of Action

As stated previously, the weight of the evidence indicates that acifluorfen does not possess mutagenic activity. Currently, no other mode of action has been hypothesized for acifluorfen.

## VIII. Structural Activity Relationships (SAR)

As a member of the diphenyl ether chemical family, acifluorfen (lactofen's major metabolite) is structurally related to four other chemicals, namely lactofen, nitrofen, oxyfluorfen, and fomesafen. Lactofen produces hepatocellular adenomas and carcinomas in mice and increases in liver neoplastic nodules and foci of cellular alteration (possible precursor of tumors) in rats. Nitrofen produces hepatocellular carcinomas in mice and pancreatic carcinomas in rats, oxyfluorfen produces marginally positive liver tumors in mice but is negative in rats, and fomesafen produces hepatocellular adenomas and carcinomas in mice.

Acifluorfen contains the following basic structural features in common with other compounds causing peroxisome proliferation:

- a chlorinated benzene ring to which an ether linkage is attached,
- a terminal carboxyl group (Eacho, 1996), and
- a lipophilic backbone (Kozuka, 1991).

## VII. SUMMARY

Based on the available toxicity data, there is evidence to support that acifluorfen is a non-genotoxic hepatocarcinogen. **Tables 5 and 7** show “Summary of Peroxisomal Effects and Liver Tumor Induction in Male and Female Mice Administered Acifluorfen”. **Tables 6 and 8** show “Summary of Liver Toxicity and Tumor Induction in Male and Female Mice Administered Acifluorfen”. **Table 9** provides quantitative evaluation of peroxisomes and cytoplasmic volume (%) in male and female mice fed acifluorfen (Blazer Technical) or positive control (DINP) for 4 weeks. These data (Tables 5-9) provide supporting evidence of peroxisome proliferation as a mode of action for liver tumors induction by acifluorfen according to the criteria recommended by ILSI; these include:

1. Changes in liver morphology were observed in both rats and mice treated with acifluorfen. These effects include: dose-dependent increase in relative liver weights, and increased incidence of cellular hypertrophy, and increased number of peroxisomes as measured by electron microscopic analysis.
2. Evidence of cell proliferation as measured by increased relative liver weights and increased replicative DNA synthesis as measured by increased hepatocellular BrdU nuclear labeling in light microscopy.
3. Dosed-dependent increase in the levels of enzymes involved in peroxisomal fatty acid metabolism, especially CN-insensitive acyl (palmitoyl) CoA oxidase activities.
4. Previously, the MTARC concluded that the peroxisome proliferation is the mode of action of lactofen in inducing liver tumors in rodents. Since acifluorfen is the major metabolite of lactofen in the rodents, it is probable that acifluorfen contributes to the peroxisome proliferation and liver tumors induced by lactofen. Therefore, the mode of action data on lactofen provide support for acifluorfen.

## VIII. References

1. Eacho, P.I., Foxworthy, P.S., Lawrence, J.W., Herron, D.K., and Noonan, D.J. (1996) Common structural requirements for peroxisome proliferation by tetrazole and carboxylic acid-containing compounds. *Ann. NY Acad. Sci.* 804: 387-402.
2. Hawks, R. (2001). Sodium Acifluorfen: Request for reassessment of current cancer classification considering peroxisome proliferation-associated mode of action. BASF Registration Doc. No. 2001/5000878. BASF Corp. January 31, 2001. MRID No. 45323500.
3. IARC Technical Report No. 24: Peroxisome proliferation and its role in carcinogenesis. December, 1994.
4. Kozuka, H., Yamada, J., Horie, S, Watanabe, T., Suga, T. and Ikeda, T. (1991) Characteristics of induction of peroxisomal fatty acid oxidation-related enzymes in rat liver by drugs. *Biochem. Pharm.* 41(4):617-623.
5. Lactofen: Report of the Mechanism of Toxicity Assessment Review Committee from Robert F. Fricke to Christine Olinger, dated March 12, 2001.
6. Reevaluation of Classification of Carcinogenicity of Acifluorfen Following Science Advisory Panel (SAP) Review of Data. The HED Cancer Peer Review Committee (HED Doc. No. 007698 dated March 17, 1988).
7. Goldenthal, E.I.; Jessup, D.C.; Geil, R.G.; et al. (1979) Lifetime Dietary Feeding Study in Mice: 285-013a. (Unpublished study received Mar 29, 1979 under 707-149; prepared by International Research and Development Corp., submitted by Rohm & Haas Co., Philadelphia, Pa.; CDL:098024-A; 098025). MRID 00082897.
8. Barnett, J.; Jenkins, L.; Parent, R. (1983) Evaluation of the Potential Oncogenic and Toxicological Effects of Long-term Dietary Administration of Tackle to Fischer 344 Rats: GSRI Project No. 413-985-41. Final rept. (Unpublished study received May 16, 1983 under 359-708; prepared by Gulf South Research Institute, submitted by Rhone-Poulenc, Inc., Monmouth Junction, NJ; CDL:250289-A; 250290; 250291). MRID 00128353.

**Table 1. Genotoxicity/Mutagenicity Profile of Acifluorfen**

ASSAY	TYPE	RESULT	MRID
Ames assay –standard plate assay (Blazer, 42.8%)	Gene mutation	<b>“Weakly” Positive in TA 100 only</b>	41480101
Ames assay – standard plate and standard plate with preincubation using manufacturing use product (46%)	Gene mutation	Negative in TA 100	45393902
Ames assay –standard plate assay using highly pure material (99.5%)	Gene mutation	Negative	45393901
Ames assay –standard plate assay and standard plate with preincubation both using end-use product (25.5%)	Gene mutation	Negative both with and without preincubation	45323501
Forward gene mutation in CHO cells	Gene mutation	Negative	41480103
Forward mutation in TK <sup>+</sup> locus in mouse lymphoma cells	Gene mutation	Negative	00122739
<i>Drosophila melanogaster</i> - (Tackle 2S) White-ivory mutation Sex-linked recessive lethal  Y chromosome loss  Dominant lethal mutations  Bithorax test	Gene mutation Gene mutation  Gene mutation  Chromosome Aberrations Chromosome Aberrations	Negative Negative  <b>Positive</b>  <b>Positive</b> Negative	00122737
<i>In vivo</i> rat cytogenetic assay	Chromosome Aberrations	Negative	00122741
Dominant lethal assay in rats	Chromosome Aberrations	Negative	00122738
<i>In vitro</i> unscheduled DNA synthesis	Other mechanisms	Negative	00122742
<i>Saccharomyces cerevisiae</i> D5 mitotic recombination (Tackle 2S, 29.7%)	Other mechanisms	<b>“Weak” positive</b>	00148272
TOTAL	11 negative 4 positive		

**Table 2. Acifluorfen Toxicity Profile** (Appendix 1 presents the Executive Summaries for critical studies)

STUDY TYPE – DOSE LEVELS	NOAEL/ LOAEL (mg/kg/day)	Liver weight	Liver enzymes	Histopathology
Chronic/Carcinogenicity - Rat <b>Fischer 344</b> (1983) 0, 25, 150, 500, 2500, or 5000 ppm. (0, 1.25, 7.50, 25.0, 125, or 250 mg/kg/day based on 1 ppm=0.05 mg/kg/day) TEST material = 19.2-25.6% a.i. (MRID No. 00128353; Accession No's. 071315 through 071317 and 250289 through 250792)	25/125	<b>increased absolute and relative liver weights</b>	<b>equivocal increased level of ALP</b> at 250 mg/kg/day	<b>increased incidence of acidophilic cells in the liver</b>  No treatment-related increase in tumors
Carcinogenicity in Mice <b>B6C3F1</b> (1982). 0, 625, 1250, or 2500 ppm (males: 0, 119, 259, 655 mg/kg/day; females: 0, 143, 313, 711 mg/kg/day) TEST material = 20.4-23.2% a.i.  (MRID No. 00122732; Accession No's.071312, 071313, 071314, 250463, and 250464)	< 119 (LDT)	<b>increased absolute and relative liver weights</b>	Not observed at LDT	<b>p&lt;0.05-0.01 liver adenomas; liver carcinomas; combined liver adenomas &amp; carcinomas</b>  <b>p&lt;0.01 trend for adenomas (♂♀); carcinomas (♂); adenomas &amp; carcinomas combined (♂♀)</b>
Carcinogenicity in Mice <b>CD-1</b> (1979). 0, 7.5, 45, or 270 ppm (1.125, 6.75, or 40.5 mg/kg/day based on 1 ppm = 0.15 mg/kg/day) for 24 months. TEST material = 39.4-40.5% a.i. (MRID No. 00082897)	6.75/40.5	<b>increased absolute and relative liver weights</b>	<b>equivocal increased levels of ALP and SGPT.</b>	p<0.05 combined liver adenomas & carcinomas (270 ppm ♀)
Chronic Feeding Study in Dogs (1983). Tackle “2S” (Acifluorfen, purity was unspecified). 0, 20, 300, or 4500 ppm (0, 0.5, 7.5 or 112.5 mg/kg/day based on 1 ppm = 0.025 mg/kg/day) for 2 years (MRID No. 00131162; Accession No's.251297 and 251298)	7.5/112.5	<b>increased absolute and relative liver weights</b>	<b>increased level of LDH</b>	<b>increased incidence of microscopic changes in the liver</b> (congestion, brown pigment, fatty vacuoles and inflammation).
Subchronic feeding study in rats <b>Fischer 344</b> (1982). 0, 20, 80, 320, 1250, 2500 or 5000 ppm (0, 2, 8, 32, 125, 250, or 500 mg/kg/day based on 1 ppm = 0.1 mg/kg/day) TEST material = 20.4-23.6% a.i. (MRID No. 00122730; Accession No. 071308)	32/125	<b>increases in liver weights</b>	at 250 or higher increased levels of SGPT and ALP	<b>increased incidence of hypertrophy of liver</b> (including increased cellular hypertrophy, mitotic activity, individual cell death, and proliferation of oval or bile duct cells)

STUDY TYPE – DOSE LEVELS	NOAEL/ LOAEL (mg/kg/day)	Liver weight	Liver enzymes	Histopathology
<p>Subchronic feeding study in mice <b>B6C3F1</b> (1982) 0, 20, 80, 320, 1250, 2500 or 5000 ppm (0, 3, 12, 48, 187.5, 375, or 750 mg/kg/day based on 1 ppm = 0.15 mg/kg/day) for 3 months TEST material = 20.4-23.2% a.i. (MRID No. 00252826; Accession No. 071308)</p>	48/187.5	<p>At 375 mg/kg/day as test material intake: Increases in absolute and relative liver weights</p>	<p>At 375 mg/kg/day and above: Increased levels of SGPT and ALP</p>	<p><b>At 187.5 mg/kg/day: histopathologic changes</b> (fatty infiltration) of the liver</p> <p>At 375 mg/kg/day and above: histopathological changes in the liver (hypertrophy, increased mitotic activity, oval cell proliferation)</p>

**Table 3. Summary of Mechanistic Studies with Acifluorfen**

STUDY TYPE – DOSE LEVELS	Liver weight	Liver enzymes	Electron microscopy
<p>Induction of the number and size of hepatic <b>peroxisomes</b> in mice following 4 week dietary feeding (MRID 45693401).</p> <p>0, 350, 1735, or 5210 ppm Blazer (46% a.i.), test material (equivalent to 0, 92, 484, and 1346 mg/kg/day in males and 0, 139, 644, and 1939 mg/kg/day in females)</p> <p>mean daily intakes of the a.i. were 42/64, 223/296 and 619/892 mg/kg, [males/females]</p>	NA	NA	<p>Induced dose-related increase in the number, size, and area of hepatic <b>peroxisomes</b> in mice treated with <math>\geq 350</math> ppm</p> <p>Dose-related increase in total peroxisomal area in high dose groups (7.2 fold increases in males and 8.3 fold increases in females).</p>
<p><b>S-phase response</b> study in the liver of mice following 3 days, 1 week, and 2 weeks feeding (MRID45803601)</p> <p>0, 350, 1735 and 5210 ppm Blazer (46 % a.i.) (<i>i.e.</i>: 160, 800 and 2400 ppm of the active ingredient), mean daily intakes of a.i. for males/females of 40/54, 227/287 and 714/845 mg/kg/day</p>	Induced dose-related increases in absolute and relative liver weights	NA	<p>At high dose, Induced a dose-related increases in panlobular hypertrophy of hepatocytes on all treatment schedules.</p> <p>Liver hypertrophy was most pronounced in the males and peaked after 1 week of treatment.</p> <p>By 2 weeks, an increase in single cell necrosis and apoptotic cells was observed primarily in high-dose males.</p> <p>Inducted BrdU labeling in the liver, which is indicative of cell proliferation (<b>S-phase response</b>).</p>

STUDY TYPE – DOSE LEVELS	Liver weight	Liver enzymes	Electron microscopy
<p>Enzyme induction study in the liver of mice following 4 weeks feeding (MRID 45793901).</p> <p>0, 350, 1735 and 5210 ppm Blazer (46 % a.i.) (160, 800 and 2400 ppm of active ingredient) for 4 weeks. Mean daily intakes of a.i. were 36.9/54.6, 180/255 and 709/933 mg/kg/d, [males/females]</p>	NA	<p>Induced dose-dependent increase in cyanide-insensitive palmitoyl-CoA-oxidase</p> <p><b>5 - 7 fold in high dose groups</b> (males and females)</p> <p>Males: 158, 458, and 676% increase at 37, 180, and 709 mg/kg/day</p> <p>Females: 3, 316, and 807% increase at 55, 255, and 933 mg/kg/day</p>	NA
<p>Measurement of Peroxisome Proliferation in Primary Rat Hepatocytes Induced by Lactofen and Five of its Metabolites including acifluorfen (45283902)</p> <p>0.003, 0.01, 0.03, and 0.1 mM</p>	NA	<p>Acifluorfen or lactofen induced CN-insensitive palmitoyl CoA oxidase activities at 0.01 mM and above.</p>	<p>EM analysis did not indicate an increased peroxisome number.</p>

**Table 4. Comparative Metabolism of acifluorfen and lactofen in Rats**

	Dose (mg/kg)	Sex	<b>Cumulative % Radioactivity Excreted</b>							
			<b>Urine</b>				<b>Feces</b>			
			<b>24 hrs</b>	<b>48 hrs</b>	<b>72 hrs</b>	<b>96 hrs</b>	<b>24 hrs</b>	<b>48 hrs</b>	<b>72 hrs</b>	<b>96 hrs</b>
Acifluorfen (1)	116	Males				48				41
	117	Females				60				23
Lactofen (2)	125	Males	29	36	44		34	62	67	
	125	Females	19	53	56		18	35	43	
			<b>Urinary and Fecal Metabolites</b>							
			<b>Major component in Urine</b>	<b>% of Total Urinary radioactivity found (% of administered dose)</b>		<b>Major component in feces</b>		<b>% of Total Fecal radioactivity found (% of administered dose)</b>		
Acifluorfen (1)	116/117	Males/ Females	Acifluorfen	93 (50)		Acifluorfen amine Acifluorfen		60-80 (19-26) 13 (4)		
Lactofen (2)	125/125	Males/ Females	Acifluorfen	91 (46)		Lactofen Acifluorfen amine Acifluorfen		30-37 (17-20) 7 (4) 6 (3)		

(1) Excerpted from MRID 00122746; Accession No. 071321. The elimination half-life of acifluorfen was 3.7 hours in females versus 8.8 hours in males.

(2) Excerpted from Accession No. 071222; HED Doc. No. 014148

**Table 5. Summary of Peroxisomal Effects and Liver Tumor Induction in Male Mice Administered Acifluorfen**

<b>Dose (mg/kg/day) as active ingredient</b>	<b>Mean Number of Peroxisome according to size classification (relative to controls, %) MRID 45693401 (a)</b>				<b>Induction of Peroxisomal Enzyme Activities; Palmitoyl CoA oxidase MRID 45793901 (b)</b>	<b>Cell proliferation; BrdU labeling in the liver (S-phase response) MRID 45803601 (c)</b>	<b>Tumor incidence (% incidence) 18-month carcinogenicity study in mice MRID 00122732 (d) (Note 2)</b>		
	<b>1 (&lt;0.1 <math>\mu\text{m}^2</math>)</b>	<b>2 (&lt;0.3 <math>\mu\text{m}^2</math>)</b>	<b>3 (&lt;0.5 <math>\mu\text{m}^2</math>)</b>	<b>Total area of peroxisomes (note 1)</b>			<b>Adenomas</b>	<b>Carcinomas</b>	<b>Adenomas/ Carcinomas Combined</b>
0	7.8 $\pm 1.5$ (SD)	8.0 $\pm 2.0$	0.5 $\pm 0.4$	1.25 $\pm 0.18$	5.24 $\pm 0.5$	1.01 $\pm 0.3$	8/58 (14 %)	1/48 (2 %)	9/58 (16 %)
37-42	<b>11.8*</b> <b>(51%)</b> $\pm 4.1$	9.6 (20%) $\pm 1.7$	0.3 $\pm 0.2$	1.49 (19%) $\pm 0.29$	<b>8.29**</b> <b>(58%)</b> $\pm 0.57$	<b>4.07 ** (304%)</b> $\pm 1.86$	NT	NT	NT
119	NT	NT	NT	NT	NT	NT	<b>18/60 (30 %) *</b>	3/50 (6 %)	<b>21/60 (35 %) *</b>
180-227	<b>15.4**</b> <b>(97%)</b> $\pm 3.1$	<b>18.7**</b> <b>(134%)</b> $\pm 2.3$	<b>2.9**</b> <b>(480%)</b> $\pm 1.6$	<b>3.43**</b> <b>(174%)</b> $\pm 0.63$	<b>25.29**</b> <b>(383%)</b> $\pm 2.93$	<b>14.09 ** (1300%)</b> $\pm 2.69$	NT	NT	NT
259	NT	NT	NT	NT	NT	NT	12/56 (21 %)	4/46 (9 %)	16/56 (29 %)
619-714	<b>22.3**</b> <b>(185%)</b> $\pm 9.4$	<b>35.7**</b> <b>(346%)</b> $\pm 10.1$	<b>11.4**</b> <b>(2180%)</b> $\pm 3.4$	<b>9.03**</b> <b>(622%)</b> $\pm 2.54$	<b>35.41**</b> <b>(576%)</b> $\pm 2.49$	<b>20.61** (1947%)</b> $\pm 3.57$	<b>25/59 (42 %) **</b>	<b>15/44 (34 %) **</b>	<b>40/59 (68 %) **</b>

NT: Not tested \* Significantly different than control ( $p < 0.05$ ) \*\* Significantly different than control ( $p < 0.01$ )Note 1: The area occupied by peroxisomes was expressed in relation to cytoplasmic region as %. Size classes 1-5 (<0.1 - >0.75  $\mu\text{m}^2$ ).Note 2: Significance of pairwise comparison with control (Fisher's Exact Test) denoted at Dose level. Tumor bearing animals/animal at risk (i.e., the animals, which died prior to the week of the first tumor occurrence for each tumor type, are removed from the animals at risk).

a) 4-week treatment at 42, 223, and 619 mg/kg/day

b) 4 week treatment at 37, 180, and 709 mg/kg/day

c) 1 week treatment at 40, 227, and 714 mg/kg/day

d) 18 month study. Overall mean doses were 119, 259, and 655 mg/kg/day. SD: Standard deviation

**Table 6. Summary of Liver Toxicity and Tumor Induction in Male Mice Administered Acifluorfen**

<b>Dose (mg/kg/day) as active ingredient</b>	<b>Subchronic study (a) (MRID 00252826)</b>		<b>Cell Proliferation Study (b) (MRID 45803601)</b>					<b>18-month carcinogenicity study in mice (MRID 00122732)</b>		
	<b>Fatty infiltration of liver</b>	<b>hypertrop hy, mitotic activity and increased levels of SGPT and ALP</b>	<b>Hypert rophy</b>	<b>Single cell necrosis and apoptotic cells</b>	<b>Body weight, g (% of controls)</b>	<b>Absolute Liver weight, g (% of controls)</b>	<b>Relative Liver weight (%of controls)</b>	<b>Body weight, % of controls</b>	<b>Liver weight, g (% of controls)</b>	<b>Adenomas/ Carcinomas Combined</b>
0					30.6	1.51	4.94		2.03	9/58 (16 %)
40-48	NF	NF	NF	NF	30.5 (99.7%)	<b>1.63 (108%)**</b>	<b>5.34 (108)**</b>	NT	NT	NT
119	NT	NT	NT	NT	NT	NT	NT	<b>90**</b>	2.41 (119%)	<b>21/60 (35 %) *</b>
188	+++ (d)	NF	NT	NT	NT	NT	NT	NT	NT	NT
227	NT	NT	NF	NF	31.3 (102%)	<b>2.13 (141%)**</b>	<b>6.80 (137%) **</b>	NT	NT	NT
259	NT	NT	NT	NT	NT	NT	NT	<b>87**</b>	<b>2.95 ** (145%)</b>	16/56 (29 %)
375	+++	+++	NT	NT	NT	NT	NT	NT	NT	NT
655-750	+++	+++	+++	+++	<b>27.3 (89%) **</b>	<b>1.90 (125%)**</b>	<b>6.94 (140%) **</b>	<b>75**</b>	<b>3.75 ** (185%)</b>	<b>40/59 (68 %) **</b>

NT: Not tested      NF: Not found      \* Significantly different than control (p<0.05)      \*\* Significantly different than control (p<0.01)

a) Treated for 90-days at 3, 12, 48, 188, 375, and 750 mg/kg/day.

b) Treated for 2 weeks at 40, 227, and 714 mg/kg/day

c) 18 month study. Overall mean doses were 119, 259, and 655 mg/kg/day. d) +++ means positive responses.

**Table 7. Summary of Peroxisomal Effects and Liver Tumor Induction in Female Mice Administered Acifluorfen**

<b>Dose (mg/kg/day) as active ingredient</b>	<b>Mean Number of Peroxisome according to size classification (relative to controls, %) MRID 45693401 (a)</b>				<b>Induction of Peroxisomal Enzyme Activities; Palmitoyl CoA oxidase MRID 45793901 (b)</b>	<b>Cell proliferation; BrdU labeling in the liver (S-phase response) MRID 45803601 (c)</b>	<b>Tumor incidence (% incidence) 18-month carcinogenicity study in mice MRID 00122732 (d) (Note 2)</b>		
	<b>1 (&lt;0.1 <math>\mu\text{m}^2</math>)</b>	<b>2 (&lt;0.3 <math>\mu\text{m}^2</math>)</b>	<b>3 (&lt;0.5 <math>\mu\text{m}^2</math>)</b>	<b>Total area of peroxisomes (note 1)</b>			<b>Adenomas</b>	<b>Carcinomas</b>	<b>Adenomas/ Carcinomas Combined</b>
0	9.5 $\pm 2.8$ (SD)	8.2 $\pm 0.7$	0.4 $\pm 0.3$	1.27 $\pm 0.13$	5.65 $\pm 0.94$	1.02 $\pm 0.24$	1/55 (1 %)	0/45 (0 %)	1/55 (2 %)
54-55					5.81 (+3%) $\pm 0.54$	<b>1.31* (28%)</b> $\pm 0.31$	NT	NT	NT
64	<b>13.2*</b> <b>(39%)</b> $\pm 1.4$	7.5 $\pm 0.8$	0.2 $\pm 0.2$	1.25 $\pm 0.18$					
143	NT	NT	NT	NT	NT	NT	5/59 (5 %)	1/47 (2 %)	6/59 (10 %)
255-313	12.1 (27%) $\pm 2.1$	<b>15.9**</b> <b>(94%)</b> $\pm 1.5$	<b>2.2**</b> <b>(450%)</b> $\pm 1.0$	<b>2.81**</b> <b>(121%)</b> $\pm 0.37$	<b>17.84**</b> <b>(+216%)</b> $\pm 1.79$	<b>3.68** (259%)</b> $\pm 0.70$	4/57 (4 %)	1/44 (2 %)	5/57 (9 %)
711	NT	NT	NT	NT	NT	NT	<b>19/58 (19 %) **</b>	<b>5/46 (11 %) *</b>	<b>24/58 (41 %) **</b>
845-933	14.6 (54%) $\pm 5.9$	<b>35.7**</b> <b>(335%)</b> $\pm 11.4$	<b>14.0**</b> <b>(3400%)</b> $\pm 3.9$	<b>10.6**</b> <b>(735%)</b> $\pm 2.95$	<b>45.57**</b> <b>(+707%)</b> $\pm 3.65$	<b>6.19** (505%)</b> $\pm 1.37$	NT	NT	NT

NT: Not tested \* Significantly different than control ( $p < 0.05$ ) \*\* Significantly different than control ( $p < 0.01$ ) SD: Standard deviation

Note 1: The area occupied by peroxisomes was expressed in relation to cytoplasmic region as %. Size classes 1-5 (<0.1 - >0.75  $\mu\text{m}^2$ ).

Note 2: Significance of pairwise comparison with control (Fisher's Exact Test) denoted at Dose level. Tumor bearing animals/animal at risk (i.e., the animals, which died prior to the week of the first tumor occurrence for each tumor type, are removed from the animals at risk).

a) 4 week treatment at 64, 296, and 892 mg/kg/day.

b) 4 week treatment at 55, 255, and 933 mg/kg/day

c) 1 week treatment at 54, 287, and 845 mg/kg/day

d) 18 month study. Overall mean doses were 143, 313, and 711 mg/kg/day.

**Table 8. Summary of Liver Toxicity and Tumor Induction in Female Mice Administered Acifluorfen**

Dose (mg/kg/d) as active ingredient	Subchronic study (a) MRID 00252826		Cell Proliferation Study (b) (MRID 45803601)					18-month carcinogenicity study in mice [c] (MRID 00122732)		
	Fatty infiltration of liver	hypertrophy, mitotic activity and increased levels of SGPT and ALP	Hypertrophy	Single cell necrosis and apoptotic cells	Body weight, g (% of controls)	Absolute Liver weight, g (% of controls)	Relative Liver weight (%of controls)	Body weight, % of controls	Liver weight, g (% of controls)	Adenomas/ Carcinomas Combined
0					25.6	1.3	5.07		1.70	1/55 ( 2 %)
48-54	NF	NF	NF	NF	25.6	1.31	5.14 (101%)	NT	NT	NT
143	NT	NT	NT	NT	NT	NT	NT	<b>89**</b>	1.59 (96%)	6/59 (10 %)
188	+++ <sup>(d)</sup>	NF	NT	NT	NT	NT	NT	NT	NT	NT
287-313	NT	NT	NF	NF	25.5	<b>1.62 (125%) **</b>	<b>6.35 (125%)**</b>	<b>78**</b>	<b>1.90 (112%)*</b>	5/57 ( 9 %)
375	+++	+++	NT	NT	NT	NT	NT	NT	NT	NT
711-845	+++	+++	+++	NF	24.8 (97%)	<b>2.19 (168%) **</b>	<b>8.81 (174%) **</b>	<b>66**</b>	<b>2.32 (136%) **</b>	<b>24/58 (41 %) **</b>

NT: Not tested    NF: Not found    \* Significantly different than control (p<0.05)    \*\* Significantly different than control (p<0.01)

a) Treated for 90-days at 3, 12, 48, 188, 375, and 750 mg/kg/day.

b) Treated for 2 weeks at 54, 287, and 845 mg/kg/day

c) 18 month study. Overall mean doses were 143, 313, and 711 mg/kg/day.

d) +++ means positive responses.

**TABLE 9. Quantitative evaluation of peroxisomes and cytoplasmic volume (%) in male and female mice fed Blazer Technical (acifluorfen) or DINP (positive control) for 4 weeks.**

Test Material	Diet conc. (ppm)	Size Classification (males)						Size Classification (females)					
		1	2	3	4	5	Total Area of peroxisomes (in %)	1	2	3	4	5	Total Area of peroxisomes (in %)
Blazer Technical <sup>a</sup>	0	7.8	8.0	0.5	0.0	0.0	1.25	9.5	8.2	0.4	0.0	0.0	1.27
	350	11.8*	9.6	0.3	0.1	0.0	1.49	13.2*	7.5	0.21	0.0	0.0	1.25
	1735	15.4**	18.7**	2.9**	0.2*	0.0	3.43**	12.1	15.9**	2.2**	0.2	0.0	2.81**
	5210	22.3**	35.7**	11.4**	2.8**	0.3**	9.03**	14.6	35.7**	14.0**	4.3**	1.0*	10.6**
DINP <sup>b</sup>	0	12.1	10.7	1.0	0.1	0.1	1.93	24.6	12.1	1.4	0.1	0.0	2.48
	500	18.2*	11.0	1.3	0.0	0.0	2.16	22.3	9.3	0.5	0.0	0.0	1.88
	1500	55.0**	15.5	0.8	0.1	0.0	3.56**	32.4	19.9**	1.2	0.0	0.0	3.63*
	4000	49.6**	47.3**	10.9**	1.1**	0.1	10.6**	31.3	37.2**	11.1**	2.1**	0.1	9.52**
	8000	18.3**	35.5**	27.9**	13.9**	2.1**	18.7**	26.4	35.5**	18.6**	9.7**	1.8	14.8**

<sup>a</sup>Data from pages 28-29 MRIID 45693401

<sup>b</sup>Data from page 29 of MRIID 45686501

\*p≤0.05; \*\*p≤0.01

## APPENDIX I

### Supporting Executive Summaries

#### Subchronic toxicity in rats

In a 90-day feeding study (MRID No. 00122730; Accession No. 071308), Tackle (20.4-23.6% a.i.) was administered to Fischer 344 rats (30/sex/dose) in the diet at levels of 0, 20, 80, 320, 1250, 2500 or 5000 ppm (0, 2, 8, 32, 125, 250 or 500 mg/kg/day based on a conversion factor of 1 ppm = 0.1 mg/kg/day) for 3 months. Body weights for 2500 and 5000 ppm males and 5000 ppm females were decreased (92.3-95.5%, 65.3%-71.2% and 83.3-90.6% of the controls, respectively). Food consumption for the 5000 ppm males and females was decreased throughout most of the study period. At 2500 ppm or above in one or both sexes, there were significant ( $p < 0.05$ ) changes in hematology parameters (erythrocyte counts, hematocrit, and hemoglobin concentration), clinical chemistry values (serum electrolytes, calcium, phosphorus, glutamic pyruvic transaminase and alkaline phosphatase activities, BUN, creatinine, serum protein, albumin and globulin concentrations) and urinalysis parameters (nitrate and urobilinogen content). Liver and kidney weights (both absolute and relative) were increased. Histopathological changes were seen in the liver of 2500 ppm or above males and females (including increased cellular hypertrophy, mitotic activity, individual cell death, and proliferation of oval or bile duct cells).

In the 1250 ppm group (in one or both sexes), there were decreases in hematology parameters (erythrocyte counts and hematocrit values), increase in absolute and relative liver weights (20-21% and 22-74%, respectively), increase in absolute and relative kidney weights (11-13% and 10-12%, respectively), and increased incidence of hypertrophy of liver cells when compared to the controls.

**No significant treatment-related effects were seen in males or females of 320 ppm or below. The LOAEL for subchronic toxicity is 1250 ppm (125 mg/kg/day) based on decreases in hematology parameters, increases in liver and kidney weights, and increased incidence of hypertrophy of liver cells when compared to the controls. The NOAEL for systemic toxicity is 320 ppm (32 mg/kg/day).**

NOTE: In the original DER [TXR No. 003409 (same as TXR No. 003556)], this study was classified as supplementary. The registrant was requested to submit the following: a) Individual animal histopathology on the animals of the 20-2500 ppm groups; b) An explanation of why in the "author's report the least frequent clinical observation was "loss of hair" while the submitted daily record of clinical signs did not mention this event [pages 98-102 of the report].

In the Agency's response to registrant's comment on previous review, this study was classified as supplementary (TXR No. 003963). The explanation of regarding "loss of hair" was satisfactory but the individual animal histopathology on the animals of the 20-2500 ppm groups was not submitted. This study is classified as Unacceptable/guideline but upgradable. The data in this study was used to set the doses in the rat chronic/carcinogenicity study.

#### Subchronic toxicity in mice

In a 90-day feeding study (MRID No. 00252826; Accession No. 071308), Tackle (20.4-23.2% a.i.) was administered to B6C3F1 mice (30/sex/dose) in the diet at levels of 0, 20, 80, 320, 1250 or 2500 ppm (0, 3, 12, 48, 187.5 or 375mg/kg/day based on a conversion factor of 1 ppm = 0.15 mg/kg/day) for 3 months. Changes in mean body weights, hematology (total white blood cell numbers, MCV, reticulocyte counts), clinical chemistry (SGPT, alkaline phosphatase, serum glucose), liver weights (absolute and relative), and histopathological changes in the liver (hypertrophy, increased mitotic activity, individual cell death and focal necrosis) were noted in males and females at the 2500 and 5000 ppm doses at 30 and 90 days. Fatty infiltration of the liver was observed in males and females at the 1250, 2500 and 5000 ppm doses at 30 days and at the 1250 and 2500 doses at 90 days.

**The NOAEL for systemic toxicity is 320 ppm (48 mg/kg/day) and the LOAEL is 1250 ppm (187.5 mg/kg/day) based on histopathologic changes (fatty infiltration) of the liver.**

This study is classified as unacceptable/guideline but upgradable. The data in this study were used to select the doses in the mouse chronic/carcinogenicity study.

#### Chronic toxicity in dogs

In a chronic toxicity study (MRID No. 00131162; Accession No's. 251297 and 251298), Tackle "2S" (Acifluorfen, sodium salt; purity was unspecified) was administered to Beagle dogs (8/sex/dose) in the diet at levels of 0, 20, 300 or 4500 ppm (0, 0.5, 7.5 or 112.5 mg/kg/day based on a conversion factor of 1 ppm = 0.025 mg/kg/day) for 2 years.

Body weights were lower than respective controls in males (-8 to -20%) and females (-9 to -10%) at 4500 ppm (high-dose) throughout the study (statistical analysis was not performed). In addition, increases in liver and kidney weights (both absolute and relative) were seen in high dose males and females. Additionally, in both sexes at high dose, red blood cell counts, hemoglobin, hematocrit and cholesterol values were significantly lower and leukocyte counts and urinary volume were significantly higher than those of the controls. In males at high dose, serum level of creatinine was lower than that of the

controls. Platelet counts, lactate dehydrogenase activity and specific gravity of urine were higher than respective controls. In females at high dose, serum levels of calcium and albumin were lower when compared to the controls. Histologically, there were increased incidence of microscopic changes in the liver (congestion, brown pigment, fatty vacuoles and inflammation) when compared to the controls.

**The LOAEL for systemic toxicity is 4500 ppm (112.5 mg/kg/day) based on decreased body weight gain, increased absolute and relative liver and kidney weights, changes in hematology, biochemistry, and urinalysis parameters and increased incidence of microscopic changes in the liver (congestion, brown pigment, fatty vacuoles and inflammation). The NOAEL for systemic toxicity is 300 ppm (7.5 mg/kg/day).**

This chronic feeding study in dogs is **classified as Unacceptable/guideline and does not satisfies the guideline data requirement for a chronic toxicity study (83-1) in dogs. However, it can be upgraded.** Reasons for this classification are the purity of the test substance was not adequately identified in the report and stability of the test substance in dog chow was not reported. However, **a new study is not required** because when this study and the data of another chronic feeding study in dog (MRID No. 00107484) with Blazer are analyzed together, they provide a reasonable understanding of the chronic toxicity of acifluorfen in dogs. Another chronic feeding study in dog (MRID No. 00107484) with Blazer is an old study (reported in 1978) which was classified as core guideline data, however, the DER for this study was not available. In the old study, there were changes in hematological and biochemical parameters related to the effects in the liver of the treated dogs at high dose (1800 to 5400 ppm) when compared to the controls. Histopathologically, treatment related alterations were observed in the liver, kidney, gall bladder and eyes when compared to the controls. The NOAEL for systemic toxicity was 50 ppm and LOAEL was 300 ppm (mid dose) based on a coagulation effects when compared to the controls (HED Doc. No. 001099; extracted from the earlier review of A. Arce dated May 2, 1979).

#### Chronic toxicity/carcinogenicity in mice [feeding]

- (1) In a 24-month carcinogenicity study (MRID No. 00082897), Blazer (39.4-40.5% a.i.) was administered to CR CD-1 mice (80/sex/dose) in the diet at levels of 0, 7.5, 45 or 270 ppm for 24 months (0, 1.125, 6.75 or 40.5 mg/kg/day based on a conversion factor of 1 ppm = 0.15 mg/kg/day). The highest dose level was initially administered to mice at a dose of 1.25 ppm on study weeks 1 to 16 before being increased to 270 ppm. Blazer produced a statistically significant ( $p < 0.05$ ) increase in the total number of liver tumors (primarily adenomas) in high dose (270 ppm) female mice. No significant increase in liver tumors occurred in male mice.

In high dose males, there was a dose-related increase in absolute and relative liver weights and relative kidney weights. In addition, there was dose related elevation of alkaline phosphatase and serum glutamic pyruvic transaminase activities in mid and

high dose male mice. However, due to the variability of the standard deviation for these parameters, statistical analysis was not performed.

**The NOAEL for systemic toxicity is 45 ppm (6.75 mg/kg/day) and the LOAEL is 270 ppm (40.5 mg/kg/day) based on increased absolute and relative liver weights, and increased relative kidney weights. It is noted that increased levels of enzymes (ALP and SGPT) seen at 40.5 mg/kg/day in this study were considered equivocal because of large standard deviations.**

**This study by itself does not satisfy the guideline requirement for a carcinogenicity study (83-2b) in mice. The deficiencies of the study report are as follows: resolution of histopathology questions; explanation of why the 270 ppm diet was analyzed only once; provide stability of the compound in feed data; explanation of Table 4 (Food Consumption). However, the requirement for carcinogenicity study for mice is satisfied when considered together with the other mouse carcinogenicity study (MRID No. 00122732; Accession No. 071312, 071313, 071314, 250463, and 250464). When the data of these two studies are analyzed together, they provide a reasonable understanding of the carcinogenicity of acifluorfen on mice.**

- (2) In a 18-month carcinogenicity study (MRID No. 00122732; Accession No's. 071312, 071313, 071314, 250463, and 250464), Tackle (20.4-23.2% a.i.) was administered to B6C3F1 mice (60/sex/dose) in the diet at levels of 0, 625, 1250 or 2500 ppm (0, 119, 259 or 655 mg/kg/day for males and 0, 143, 313 or 711 mg/kg/day for females) for 18 months.

An increase in mortality was seen in high dose males (control, 1/60; high-dose, 10/60). Body weights in all treated mice were reduced when compared to controls. Beginning at week 2 for mid- and high-dose males and week 6 for low-dose males, body weights were significantly reduced ( $p < 0.01$ ) relative to controls. The body weight decrease for low-, mid-, and high-dose males was 8%, 10% and 23% of the controls, respectively at week 13 and 10%, 13% and 25% of the controls, respectively at week 79. Similar results were obtained with females, except that reduced body weights of low- and mid-dose females were not significant until week 13. The body weight decrease for low-, mid-, and high-dose females was 6%, 5% and 14% of the controls, respectively at week 13 and 11%, 22% and 34% of the controls, respectively at week 79. Mean food consumption in high-dose males was higher relative to the controls most of the study period.

Mean corpuscular volume (MCV) and segmented neutrophil counts were decreased and lymphocyte and RBC counts were increased compared to controls in all treated males at final sacrifice. Segmented neutrophil counts was decreased and lymphocyte counts were increased in all treated females (at interim sacrifice) and in mid- and high-dose females (at final sacrifice) when compared to controls.

Mean absolute and relative liver weights of treated males and females were greater than the controls at interim and final sacrifice. Gross pathology showed an apparent dose-related increase in incidence of liver masses in treated males when compared to the controls at final sacrifice. In high dose females, an increase in incidence (37%) of liver masses was observed. The incidence of white foci (1 mm) on the nonglandular portion of the stomach were seen in high-dose males and mid- and high-dose females. In addition, one high-dose male and one high-dose female each had an ulcer of the stomach.

Acifluorfen was associated with statistically significant positive trends for liver tumors (adenomas, carcinomas, and adenomas/carcinomas combined) and stomach tumors (papillomas) in both sexes. The liver tumors were significantly increased above the controls at the lowest dose level tested (625 ppm) in male mice and at the highest dose level tested (2500 ppm) in both sexes. In addition, the stomach tumors were significantly increased above the controls at the highest dose level tested (2500 ppm) in both sexes. The highest dose tested was considered adequate in evaluation of carcinogenic potential of the test chemical.

**The NOAEL for systemic toxicity is not established. The LOAEL for systemic toxicity is equal or lower than 625 ppm (119 and 143 mg/kg/day for males and females, respectively) based on reduced body weight, increased absolute and relative liver weights, and changes in hematologic parameters (decreased MCV counts, decreased segmented neutrophil counts, increased RBC counts, and increased lymphocyte counts).** This study is classified as Acceptable/Guideline and satisfies the Subdivision F guideline requirement for a carcinogenicity study (83-2b) in mice.

#### Chronic toxicity/carcinogenicity in rats [feeding]

In a two-year feeding/carcinogenicity study (MRID No. 00128353; Accession No's. 071315 through 071317 and 250289 through 250792), Tackle (19.1-25.6% a.i.) was administered to Fischer 344 rats (73/sex/dose) in the diet at levels of 0, 25, 150, 500, 2500 or 5000 ppm for 2 years (0, 1.25, 7.50, 25.0, 125 or 250 mg/kg/day based on a conversion factor of 1 ppm=0.05 mg/kg/day).

All males and 61/65 females in 5000 ppm group died before termination of the study. Mean body weight was significantly decreased in males at the 2500 (7-9%) and 5000 ppm (18-35%) throughout the study relative to the controls. In females, the body weight was also decreased in 2500 (6-16%) and 5000 ppm (11-28%) groups relative to the controls.

Red cell counts, hematocrit and hemoglobin values were significantly lower in the 5000 ppm males when compared to the controls.

In the 5000 ppm males and females, blood glucose, triglyceride, and serum globulin and total protein were significantly lower than those of the control animals; BUN, creatinine and alkaline phosphatase were significantly higher than those of the control animals. In the 2500 ppm males, blood glucose, triglyceride and globulin were significantly lower and BUN was significantly higher when compared to the control animals. In the 2500 ppm females, triglyceride and globulin were significantly lower and alkaline phosphatase was significantly higher when compared to the control animals.

Gross necropsy showed that there was an increased incidence of kidney and liver discoloration, stomach ulcers and reduced testes size for the 5000 ppm males when compared to the controls. In females, an increased incidence of kidney lesions (distended pelvis/calculi) was observed in the 2500 and 5000 ppm animals and stomach ulceration was observed in the 5000 ppm animals when compared to the controls.

In 2500 ppm males, there were increases in absolute and relative liver weights, decreases in spleen weights, and increases in relative kidney weights. In 5000 ppm males, there were increases in liver weights and decreases in spleen weights. In females at 2500 and 5000 ppm, there were increases in relative liver weights and decreases in heart weights.

Histologically, there were increased incidences of acidophilic cells in the liver of the 5000 ppm group in both sexes and in the 2500 ppm females. In addition, there were increased incidences of nephritis/pyelonephritis in 2500 and 5000 ppm group females. An increase in the incidence of stomach ulcer was seen in 5000 ppm males and females. Testicular atrophy was also seen in 5000 ppm males.

**Under the condition of the study, no treatment-related increase in tumor incidence was found in the acifluorfen treated rats.**

**The NOAEL for systemic toxicity is 500 ppm (25 mg/kg/day); the LOAEL is 2500 ppm (125 mg/kg/day) based on reduced body weight, increased absolute and relative liver weights and increased kidney weights, increased incidence of nephritis/pyelonephritis, increased incidence of acidophilic cells in the liver, and related changes in clinical chemistry parameters**

The highest dose (5000 ppm) tested was considered excessively toxic in evaluation of carcinogenic potential of the test chemical based on findings of reduced body weights, increased mortality, increased liver weights and liver enzyme changes (alkaline phosphatase), renal changes (nephritis and pyelonephritis), stomach ulcers and decreased testes size. This study is classified as Acceptable/guideline and satisfies the guideline data requirement for a combined chronic/carcinogenicity study (83-5) in rats.

Another rat chronic feeding study (MRID No. 00087478) with Blazer was also conducted by Rohm and Haas. The DER (HED Doc. No. 001099 and 001251) for this study indicated no increase in any tumor incidence in treated animals relative to the controls. However, the experimental design of the study used multiple shifts in dose levels at different durations of the study. It is difficult to establish at what dose levels or times that changes in biological parameters occurred. However, when the data of these two studies are analyzed together, they provide a reasonable understanding of the chronic toxicity of acifluorfen in rats.